Efficient microwave-assisted synthetic protocols and in silico behaviour prediction of per-substituted β-cyclodextrins

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Efficient microwave-assisted synthetic protocols and *in silico* behaviour prediction of *per*-substituted β-cyclodextrins.

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Selective *per*-substituted cyclodextrin design enables the carrier's physicochemical and binding properties to be tailored and can even modify some biological native structure effects. We herein report a number of highly efficient microwave-assisted synthetic protocols for the preparation of several amino, ureido and thioureido *per*-substituted β-cyclodextrin derivatives. A rapid parallel synthetic approach has given a set of 14 different CD derivatives. Our strategy is supported by computational analyses which were used to estimate the physicochemical behaviour of *per*-substituted derivatives and tailor suitable substituents.

Introduction

Cyclodextrins (CDs) are natural cyclic oligosaccharides composed of α-(1-4)-linked glucose units and which feature a relatively rigid troncoconic structure.[1] The salient characteristic of a CD molecule is the presence of a central "cavity" or "hole" which provides an excellent resting site for hydrophobic molecules of appropriate dimensions.[2] Modified CDs have been the subject of intense study in recent years. The cyclic maltooligosaccharide nucleus combines biocompatibility, availability and a tubular symmetric framework with well differentiated faces that can be modified in a flexible manner. The selective substitution of native CDs dramatically affects their physicochemical properties, and so their behaviour in aqueous media can be tailored to specific needs.[3] The synthesis of selective mono-, poly- and *per*-substituted CDs has often been limited by poor overall yield and time-consuming purification procedures. In particular, full derivatisation generally gives mixtures of poly-substituted products with varying degrees of substitution.

Per-(6-amino-6-deoxy)-β-CDs are an important class of derivatives because they are well suited to a high number of applications and lend themselves particularly well to use as biomimetic receptors. Amino-*per*-substitution on the primary face usually preserves the symmetry and the hydrophobic character of the CD cavity. Notably, chemically modified CDs that bear cationic groups on one rim may boast spatial orientation capabilities and may potentially self-assemble into discrete architectures, tubular assemblies and nanometric objects.[4] Tubular assemblies, in particular, have attracted a great deal of attention because they act as ion channels in the mediation of anion over cation transport.[5] In fact, a recent study showed that *per*-amino β-CD derivatives mimic an antibacterial peptide, called polymyxin B, and strongly permeabilize bacterial membranes and inhibit bacterial proliferation.[6] Supramolecular adducts obtained from cationic CDs have been programmed to complex, compact, deliver and release plasmid DNA into a target cell.[7] *Per*-amino β-CD derivatives and their ability to overcome *in vitro* protein aggregation have been the focus of further studies which have led to them potentially finding biomedical applications ranging from disease treatment (such as Alzheimer's and Parkinson's diseases) to the stabilising, storage and delivery of protein drugs.[8] Antitoxin activity has also been ascribed to the complementary electrostatic interactions between cationic substituents on the primary hydroxyl group CD rim and the negatively charged amino acids on the inner surface of the protective antigen pore.[9]

It has been demonstrated that *6-per*-amino CDs posses lower haemolytic activity than native CDs,[10] although both are characterized by poor membrane permeability.[8b]. This drawback has prompted investigations into CD derivatisation that may enhance membrane permeability, a mandatory requirement for drug delivery.

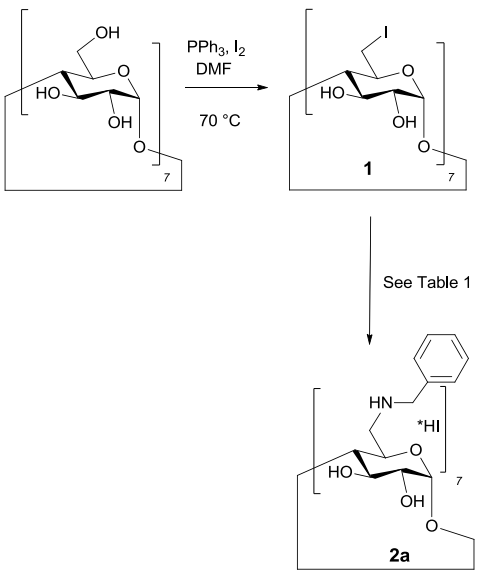
The aim of this work is the optimization of reliable non-conventional procedures for the synthesis of *per*-substituted CDs that bear amino groups on the primary face. This important scaffold may exploit multiple electrostatic interactions *via* its protonable *N*-sites. We have therefore looked to broaden the existing CD library by means of synthetic protocols in which low-boiling amines are efficiently employed. In this work, these *per*-amino β-CDs were used as building blocks for the preparation of reactive intermediates (isocyanate and isothiocyanate) to afford more lipophilic ureido and thioureido CD derivatives. Microwave (MW) and ultrasound (US) irradiation were exploited as a useful enabling technique for the synthesis of tailor-made selectively modified CDs.[11] A computational study was carried out to aid the design of a set of *per*-substituted CDs that express a wide range of physicochemical properties. We applied VolSurf+[12], a computational tool designed to produce descriptors related to physical and chemical properties from 3D molecular field maps. In the standard procedure, GRID interaction fields[13] were calculated around the target molecules. VolSurf+ compresses the information present in 3D grid maps into a few 2D numerical descriptors which are simple to understand and to interpret. Finally, Volsurf+ descriptors provided a global depiction of the chemical structure, in terms of size and shape, of both the hydrophilic and hydrophobic regions of the CDs and to the balance between them. Two synthetic methodologies, compatible with molecular diversity oriented strategies, were pursued with the aim of modulating the structural (sizes and shapes) and functional (surfaces chemistry and charges) properties of CD derivatives. Rapid and parallel synthesis of amino, ureido and thioureido *per*-substituted CD derivatives were optimized and the synthesis of a set of 14 different CDs derivatives is herein described.

Results and Discussion

Cationic *hepta*-substituted β-CD derivatives were synthesized via the nucleophilic displacement of *heptakis*(6-iodo-6-deoxy)-β-CD with amines. As depicted in Scheme 1, the selective replacement of all the primary hydroxyl groups on a β-CD with iodine atoms was obtained using I_2 and Ph_3P in DMF, in accordance with the literature.[14] The following MW-assisted nucleophilic substitution

afforded the *per*-amino β-CDs in good yields, whereas the conventional reaction does not typically run to completion and leaves the problem of separating the fully heptasubstituted from the hexa- and/or pentasubstituted β-CD derivatives. Thus, quantitative conversions are required because purification reduces the isolated yield excessively. To the best of our knowledge, the solventless quantitative conversion of *per*-iodo CDs (**1**) to the corresponding amino derivative under conventional heating at 75-80 °C in excess of amine required several days.[8b] When the reaction is performed with an excess of amine in a minimum amount of solvent (DMSO, DMF) it does not proceed to completion.

Benzylamine was selected as the preliminary substrate for the optimising of reaction conditions. It was thus reacted with *per*-(6-iodo-6-deoxy)-β-CD in DMF in MW at 85°C. Dielectric heating dramatically cut reaction time and the desired product was isolated by precipitation in acetone. The reaction yields were comparable in monomode or multimode MW[15] reactors. When the reaction was repeated with 50 eq of benzylamine the reaction did not proceed to completion and a mixture of poly-substituted CD derivatives was isolated.



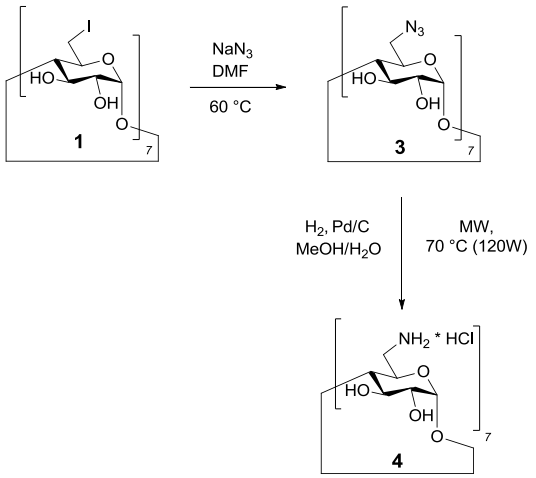
Scheme 1 Synthesis of *per*-(6-benzyl-6-deoxy)-β-CD (**2a**)

Table 1: Nucleophilic substitution of *per*-(6-iodo-6-deoxy)-β-CD with benzyl amine.

Entry	Amine eq. (ratio amine:solvent)	Yield
1	436 (1:1)	65%[a], 68%[b,c]
2	150 (1:5)	62%[b]
3	100 (1:4)	60%[b]
4	50 (1:8)	-[b,d]

a) Multimode oven (MicroSYNTH - Milestone) 85°C, 150 W; b) Monomode reactor (Discovery - CEM) 85°C, 150 W; c) Multimode reactor (SynthWAVE - Milestone). N_2 (20 bar) 85°C, 150 W; d) The product was obtained as a mixture of poly-substituted β-CD derivatives

The preparation of *per*-(6-amino-6-deoxy)-β-CD (**4**) *via* the per-(6-azido-6-deoxy)-β-CD derivative (**3**) was optimised with the aim of pursuing the synthesis of 6-*per*-substituted ureido and thioureido β-CD derivatives. The Staudinger reaction has already been described[16] and commonly pursued[17] even though it exacerbates the drawback of residual Ph_3PO.



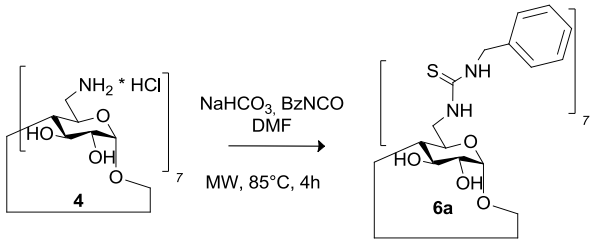
Scheme 2 Synthesis of *per*-(6-amino-6-deoxy)-β-CD

Table 2 Synthesis of *per*-(6-amino-6-deoxy)-β-CD (**4**).

Entry	Reaction Conditions	Yield	comments
1	PPh_3, NH_4OH, DMF, magnetic stirring, 25 °C, 18h	82%	a
2	N_2H_4, Pd/C, MeOH/H_2O, reflux, 85 °C, 3h	85%	b
3	Pd/C, H_2, MeOH/H_2O, SynthWave (120 W), 70 °C, 3h	91%	-

a) The final product was isolated with PPh_3O impurities (7%), b) The product was isolated with N_2H_4 impurities as demonstrated by IR analysis

As has already been described in the literature,[18] the reduction of the epta-azido derivative via catalytic hydrogenation with Pd/C is a very difficult task and so a preliminary study was carried out, as a part of this work, to investigate catalytic transfer hydrogenation with hydrazine as the hydrogen donor to avoid the presence of PPh_3O in the final product. Derivative **3** was heated under reflux in a solution of N_2H_4 in methanol/H_2O 3h and the desired amine **4** was collected in a 85% yield as the chloride salt. The presence of hydrazine in the final product, a serious drawback due to its high toxicity and instability, was detected by IR. This prompted the investigation of catalytic hydrogenation under MW irradiation in a professional MW reactor equipped with temperature and pressure control systems. A solution of *per*-(6-azido-6-deoxy)-β-CD in methanol/H_2O was irradiated at 75 °C in the presence of Pd/C under H_2 pressure (10 bar). The equipment guarantees homogenous and regular reaction mixture heating because the reaction vessel is immersed in an adsorbing medium, such as ethylene glycol. As depicted in Table 2, the catalytic hydrogenation of **3** gave the pure final product after 3 h of irradiation in 90% yield.



Scheme 3 Synthesis of *per*-(6(3-benzylthioureido)-6-deoxy)-β-CD

To obtain the ureido derivatives, the chloride salts of *per*-(6-amino-6-deoxy)-β-CD HCl was treated with sodium bicarbonate and heated in MW with benzyl isothiocyanate in DMF for 4 h. Despite the widespread use of this procedure, a mixture of different poly-substituted derivatives is generally produced under conventional conditions, as can be inferred from the lack of examples in the literature.[19] MW promoted the reaction and the desired product was recovered in satisfactory yields after reverse phase purification.

On the basis of the optimized synthetic protocols, 68 potential reagents (27 amines, 19 isothiocyanate, and 22 isocyanates, see Supplementary Information for structures) were selected on the basis of their commercial availability and substructure. An in-silico approach was used to select a set of substituents with a wide range of physicochemical properties. Volsurf+ descriptors were used to characterize the substituents and then submitted to principal component analysis (PCA) (see Computational Studies for details). PCA provides two results: the score and the loading plots. The first graphically shows similarities between objects (in this case the substituents) whereas the second suggests the interpretation of such similarities in terms of the descriptors used to characterize objects. The score plot (Figure 1) of the two main PCs (PC1 and PC2) shows how the substituents can be split into three clusters reflecting the presence of different functional groups: amino, isocyanate and isothiocyanate. In addition, sub-sets of substituents corresponding to the presence of alkyl, benzyl or phenyl moieties can be observed in each cluster.

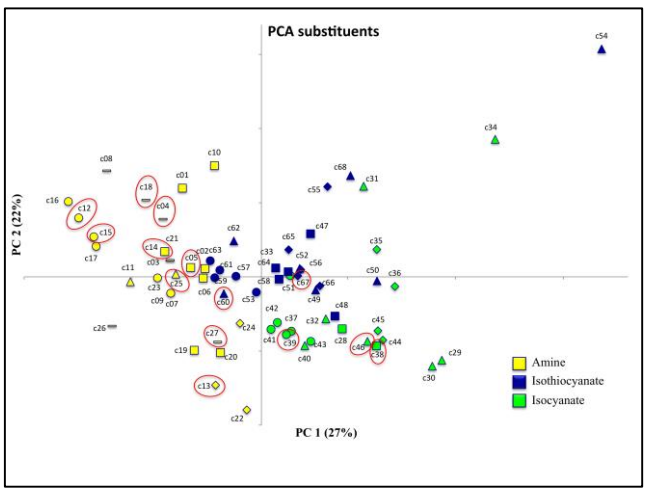


Figure 1. The score plot of the first two main PCs (in brackets the variance %). Functional groups: amines, isocyanate, isothiocyanates. See legend for symbols. ● Alkyl,■ Benzyl,♦ Phenyl, ▬ Secondary amines, ▲ Others. Synthesized products were circled in red.

Other useful information was extracted from the loadings plot (Figure 2). PCs are obtained as linear combinations of original descriptors. The loading of a single descriptor indicates how much this descriptor participates in defining the PC. Variables that contribute very little to the PCs have small loading values and are plotted around the centre of the plot, whereas the variables that contribute most are plotted around the borders of the plot. H-bond and hydrophilic Volsurf+ descriptors were essentially located in the upper part of the loadings plot while hydrophobic descriptors in the lower thus these descriptors were the variables that contribute most to PC1 and PC2. As a consequence, amines, that were located in the upper part of score plot, were characterized mainly by their H-bond donors/acceptors and hydrophilic properties whereas isocyanates, located in the lower part, mainly by their hydrophobic properties; finally, the isothiocyanates were in the middle with average properties.

These results confirmed the ability of Volsurf+ descriptors to single out substituents' physicochemical properties, while PCA permitted them to be grouped into clusters. A small but representative set of substituents can be designed by choosing some objects from each cluster. A Volsurf+ study performed using the structure of the whole CD essentially confirmed these results, but more time was required to take CD flexibility into account. Thus, there was no advantage in considering the complete structure of the CDs.

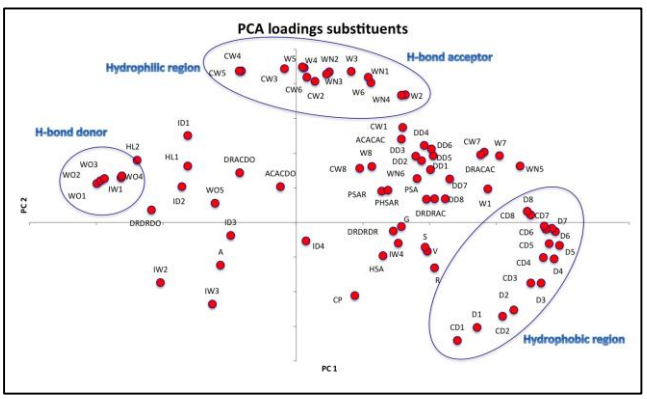


Figure 2. The loadings plot of the first two main PCs

A diversity set of 14 substituents was chosen from the PCA scores analysis (see Figure 1). As depicted in Figure 3, the selected CD derivatives belong to three series of functionalized CDs and the substituents were picked from the sub-sets corresponding to the presence of alkyl, benzyl or phenyl moieties. When amino derivatives were synthesized, primary and secondary amines were selected. In the end, 9 *per*-6-amino β-CD derivatives, 2 *per*-6-ureido β-CD derivatives and 3 *per*-6-thioureido β-CDs derivatives were synthesized.

The nucleophilic displacement of *heptakis*(6-iodo-6-deoxy)-β-CD was successfully repeated with phenylethyl amine, 2-chlorobenzylamine, aniline and with secondary amines such as 1-methyl piperazine, morpholine and tetrahydroisoquinoline. The products were recovered and purified by precipitation in 60% average yield. To obtain more hydrophylic compounds, volatile amines such as allylamine and butylamine were selected and reacted in a MW reactor SynthWAVE, (Milestone), a closed-cavity system, designed to work in a wide range of pressure (up to

200 bar) and temperatures (up to 300 °C). The reactions were all performed under N_2 pressure (20 bar) which allowed for the use of a wider range of low-boiling reagents. The combination of physical activation with MW irradiation and the MW reactor, equipped with a rack for several test tubes, facilitated the search for the optimal reaction conditions.

The synthesis of 6-*per*-ureido β-CD derivatives and 6-*per*-thioureido β-CD was repeated. Phenyl and cyclohexyl isocyanate, benzyl, butyl and phenyl ethyl isothiocyanate were reacted with *per*-(6-amino-6-deoxy)-β-CD. The reactions were performed in MW at 85°C for 2h and the products were recovered in 37% average yield after column purification. As has already been described above, volatile butylisothiocyanate was selected and reacted in a MW SynthWAVE reactor (Milestone) to obtain more hydrophylic compounds.

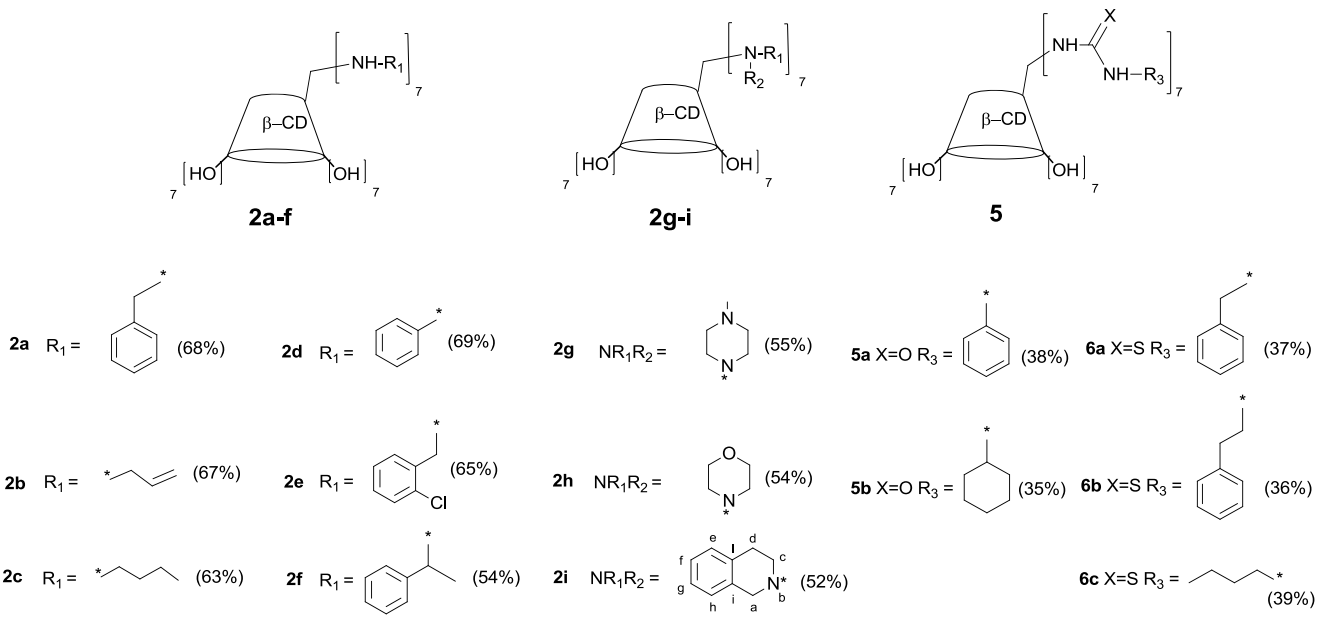


Figure 3. Molecular structures and reaction yields in bracket of the diversity set

Conclusions

In this manuscript we have described a few highly efficient MW-assisted synthetic protocols for the preparation of several amino, ureido and thioureido *per*-substituted β-CD derivatives. The structural and functional properties of the CD derivatives were designed by a preliminary computational study which expressed a wide range of physicochemical properties. Two synthetic protocols, compatible with a diversity oriented strategy, have been pursued and a set of 14 *per*-substituted CDs have been synthesized *via* MW-assisted optimized procedures. Hydrophilic CD derivatives have been obtained by performing the reaction in a closed cavity MW reactor that allows high temperature and high gas pressures to be achieved and consequently enable the use of low-boiling point reagents.

Experimental

Computational Studies

PCA is a general tool for the interpretation of large data tables in which the number of the original variables (in this case the Volsurf+ descriptors for each substituent) is reduced by projection of the objects (*i.e.* the substituents) onto a smaller number of new variables named principal components (PC). The PCs are orientated so that the first PC describes as much of the original variation between the objects as possible. The second PC is orientated in an orthogonal manner to the first PC and is directed to describe as much of the remaining variation as possible and so on. The projection of objects onto a PC is called scoring. By plotting the scores for two PCs it is possible to graphically find similarities and differences between objects. Loading plots represent the original descriptors in the PC space.

SMILES[20] codes for the 68 substituents were prepared and submitted to VolSurf+ (version1.0.4, Molecular Discovery Ltd. Pinner, Middlesex, UK, 2009, http://www.moldiscovery.com) using default settings and four probes (OH2, DRY, N1 and O probes) that mimic the compounds' water, hydrophobic, hydrogen bond acceptor and hydrogen bond donor interactions with the environment respectively.

Briefly, VolSurf+ is a computational procedure used to discover molecular descriptors from 3D molecular interaction fields (MIFs)[21] obtained using the GRID force field.[22] Interaction fields are obtained with different probes and the surfaces of the regions that encompass interaction energy values under certain cut-off limits are calculated. In particular, water (OH2), hydrophobic (DRY), hydrogen bond donor (HBD, amide N1) and hydrogen bond acceptor (HBA, carbonyl O) probes were considered in the present work. Since VolSurf+ calculates 82 descriptors (Table 3) that represent polarity and hydrophobicity (as well as size and shape) of molecules, they are generally well suited for the modelling of a wide range of molecule physicochemical properties.[21]

Table 3: Volsurf+ descriptors grouped on the basis of their physical-chemical meaning.

Block	Descriptors	Number
Size/Shape	V, S, R, G, W1, WO1, WN1	7
OH2	W2-W8, IW1-IW4.CW1-CW8	19
N1	WN2-WN6	5
O	WO2-WO6	5
DRY	D1-D8, DD1-DD8, ID1-ID4, CD1-CD8	28
Others	HSA, PSAR, PSA, HL1, HL2, A, CP, PHSAR, DRDRDR, DRDRAC, DRDRDO, DRACAC, DRACDO, DRDODO, ACACAC, ACACDO, ACDODO, DODODO	18

General Methods.

Commercially available reagents and solvents were used without further purification. β-CD was kindly provided by Wacker Chemie. Reactions were monitored by TLC on Merck 60 F254 (0.25 mm) plates. Spot detection was carried out via staining with 5% H_2SO_4 in ethanol. NMR spectra were recorded with a Bruker 300 Avance (300 MHz and 75 MHz for ^1H and ^{13}C, respectively) at 25 °C. Chemical shifts were calibrated to the residual proton and carbon resonances of the solvent; DMSO-d6 (δH = 2.54, δC = 39.5), D_2O (δH = 4.79). Chemical shifts (δ) are given in ppm, and coupling constants (J) in Hz. ESI-mass spectra were recorded on a Waters Micromass ZQ equipped with an ESI source.

MW-promoted reactions were carried out in three professional reactors; the MicroSYNTH and the SynthWAVE by Milestone and the Discover by CEM.

General procedure for the synthesis of *per*-(6-alkylamino-6-deoxy)-β-cyclodextrin

per-(6-iodo-6-deoxy)-β-CD (0.0525 mmol) was dissolved in DMF (2 mL) and amine (5.25 mmol) was added. The reaction was carried out under magnetic stirring in a MW reactor (150 W) at 85 °C for 1 h. After concentration under vacuum to half volume and the addition of acetone (10 mL), a solid product was collected by filtration on a Hirsch funnel. The desired product was recovered without further purification. When the reaction was performed in the SynthWave MW reactor, 20 bar of nitrogen pressure were applied.

***per*-(6-benzylamino-6-deoxy)-β-cyclodextrin (2a)** is a white powder; yield = 68%, ^1H NMR (300 MHz, DMSO-d_6) δ = 7.27-7.10 (m, 35H, H-Ar), 5.76-5.73 (m, O(2)H, O(3)H), 4.83 (br s 7H, H-1), 3.60-3.50 (m, 28H, Ph-C$\underline{H_2}$, H-5, H-3), 3.50-3.20 (m, overlapped with water, H-2, H-4), 2.61 (m, 14H, H-6) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 140.8 (C_{ipso}), 128 (C_{meta}), 127.6 (C_{ortho}), 126.4 (C_{para}), 102.14 (C1), 82.89 (C4), 72.91 (C3), 72.52 (C2), 70.6 (C5), 53.2 (Ph-$\underline{C}H_2$), 48.3 (C6) ppm; MS (ESI): *m/z* calcd for $C_{91}H_{119}N_7O_{28}$ $[M + H]^+$ 1758.81 found 1758.59.

***per*-(6-allylamino-6-deoxy)-β-CD (2b)** is a yellowish powder; yield = 67%, ^1H NMR (300 MHz, DMSO-d_6) δ 6-20-5.85 (m, O(2)H, O(3)H,-C$\underline{H}$=CH_2), 5.50-5.20 (m 14H, -CH=C$\underline{H_2}$), 5.09-5.05 (m, 7H, H-1), 3.93-3.70 (m, 7H, H-5), 3.70-3.25 (m, overlapped with water, H-2, H-3, H-4, NHC$\underline{H_2}$CH=CH_2), 3.15-2.95 (m, 14H, H-6) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 132.2 (C$\underline{H}$=CH_2), 120.5 (CH=$\underline{C}H_2$), 101.5 (C1), 82.3 (C4), 72.4 (C3), 71.8 (C2), 68.2 (C5), 50.65 (NH$\underline{C}H_2$CH=CH_2), 47.3 (C6) ppm; MS (ESI): *m/z* calcd for $C_{63}H_{105}N_7O_{28}$ $[M + 2H]^{2+}$ 704.85 found 705.33.

***per*-(6-butylamino-6-deoxy)-β-CD (2c)** is a yellowish powder; yield = 63%, ^1H NMR (300 MHz, DMSO-d_6) δ 6.10-5.86 (m, O(2)H, O(3)H), 5.07 (br s, 7H, H-1), 4.20-3.80 (m, 7H, H-5), 3.80-3.30 (m, 21H, H-2, H-3, H-4), 3.30-2.80 (m, 28H, H-6, C$\underline{H_2}$NH), 1.8-1.2 (m, 28 H), 1.02-0.73 (m, 21H, C$\underline{H_3}$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 101.8 (C1), 72.5 (C3), 72.3 (C2), 68.4 (C5), 48.6 (C6), 37.4 (NH-$\underline{C}H_2$-CH_2), 31.7(NH-CH_2-$\underline{C}H_2$), 20.6 (-$\underline{C}H_2$-CH_3), 14.1 ($\underline{C}H_3$) ppm; MS (ESI): *m/z* calcd for $C_{70}H_{133}N_7O_{28}$ $[M + 2H]^{2+}$ 760.96 found 761.41.

***per*-(6-anilino-6-deoxy)-β-CD (2d)** is a white powder; yield = 69%, ^1H NMR (300 MHz, DMSO-d_6) δ 7.27-7.10 (m, 35H, H-Ar), 5.03 (br s, 7H, H-1), 3.86-3.15 (m, 42 H, overlapped with water, H-2, H-3, H-4, H-5, H-6) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.2 (C_{ipso}), 129.5 (C_{meta}), 116.1 (C_{ortho}), 118.9 (C_{para}), 102.4 (C1), 86.3 (C4), 72.7 (C3), 72.4 (C2), 71.2 (C5), 49 (C6) ppm; MS (ESI): *m/z* calcd for $C_{84}H_{105}N_7O_{28}$ $[M + H]^+$ 1660.70 found 1661.01.

***per*-(6-(2-chlorobenzylamino)-6-deoxy)-β-CD (2e)** is a white powder; yield = 65%, ^1H NMR (300 MHz, DMSO-d_6) δ 7.61-7.19 (m, 28H, H-Ar), 5.86-5.80 (m, O(2)H, O(3)H), 4.99-4.82 (m, 7H, H-1), 3.81-3.66 (m, 28H, H-3, H-5, Ph-C$\underline{H_2}$), 3.55-3.38 (m, overlapped with water, H-2, H-4), 2.86 (H-6) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 138.2 (C_{ipso}), 133.2 ($\underline{C}$-Cl), 129.9 (Ph), 129.8 (Ph), 129.6 (Ph), 127.9 (Ph), 102.3 (C1), 83.2 (C4), 73.1 (C3), 72.6 (C2), 70.5 (C5), 50.1 (Ph-$\underline{C}H_2$), 48.7 (C6) ppm; MS (ESI): *m/z* calcd for $C_{91}H_{112}Cl_7N_7O_{28}$ $[M + 2H]^{2+}$ 998.77 found 998.93.

***per*-(6-phenylethylamino-6-deoxy)-β-CD (2f)** is a white powder; yield = 54%, ^1H NMR (300 MHz, DMSO-d_6) δ 7.41-7.02 (m, 35H, H-Ar), 5.87-5.77 (m, O(2)H, O(3)H), 4.99-4.75 (m, 7H, H-1), 3.85-3.17 (m, 35H, - Ph-C$\underline{H}$, H-2, H-3, H-4, H-5), 2.75-2.58 (m, 14H, H-6), 1.26-1.05 (m, 21H, C$\underline{H_3}$), ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 145.9 (C_{ipso}), 128.6 (C_{meta}), 126.6 (C_{ortho}), 126.2 (C_{para}), 101.6 (C1), 83.2 (C4), 72.5 (C3), 72.1 (C2), 70.6 (C5), 57.5 (Ph-$\underline{C}$H), 48.6 (C6) 23.9 ($\underline{C}H_3$)ppm; MS (ESI): *m/z* calcd for $C_{98}H_{133}N_7O_{28}$ $[M + H]^+$ 1856.92 found 1857.02.

***per*-(6-methyl piperazino-6-deoxy)-β-CD (2g)** is a white powder; yield = 55%, ^1H NMR (300 MHz, DMSO-d_6) δ 5.96-5.85 (m, O(2)H, O(3)H), 4.90 (br s 7H, H-1), 3.70-3.20 (m, overlapped with water, H-2, H-3, H-4, H-5), 2.80-2.25 (m, overlapped with DMSO, C$\underline{H_2}$N, C$\underline{H_3}$N, H-6) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 102.5 (C1), 84.2 (C4), 73.3 (C3), 55.3 ($\underline{C}H_2$N), 54.7 ($\underline{C}H_2$N), 54 ($\underline{C}H_2$N), 45.8 ($\underline{C}H_3$) ppm; MS (ESI): *m/z* calcd for $C_{77}H_{140}N_{14}O_{28}$ $[M + H]^+$ 1710.00 found 1710.82.

***per*-(6-morpholino-6-deoxy)-β-CD (2h)** is a white powder; yield = 54%, ^1H NMR (300 MHz, D_2O) δ 5.08 (br s, 7H, H-1), 4.22 (m, 7H, H-5), 4.01-3.87(m, 21H, H-3, C$\underline{H_2}$O), 3.67-3.59 (m, 14H, H-2, H-4), 3.26-2.99 (m, 42H, C$\underline{H_2}$N, H-6) ppm; ^{13}C NMR (75 MHz, D_2O) δ 100.2 (C1), 80.8 (C4), 72.2 (C3), 71.8 (C2), 68.1 (C5), 64.9 ($\underline{C}H_2$O), 58.1 (C6), 53.6 ($\underline{C}H_2$N) ppm; MS (ESI): *m/z* calcd for $C_{70}H_{119}N_7O_{35}$ $[M + H]^+$ 1618.77 found 1619.06.

***per*-(6-tetrahydroisoquinolino-6-deoxy)-β-CD (2i)** is a white powder; yield = 52%, ^1H NMR (300 MHz, DMSO-d_6) δ 7.22-6.76 (m, 28H, H-Ar), 6.03-5.93 (m, O(2)H, O(3)H), 4.90 (br s 7H, H-1), 4.00-3.38 (m, overlapped with water,H-2, H-3, H-4, H-5, C$\underline{H_2}$(a)N), 2.96-2.55 (m, 14H, H-6, C$\underline{H_2}$(c), C$\underline{H_2}$(d)) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 136.1 (Ci), 134.2 (Cl), 128.8, 127.8,

127.2, 126.3 (Ce-h), 102.8 (C1), 83.9 (C4), 73.4 (C3), 72.9 (C2), 70.9 (C5), 57.3 (Cc), 56.8 (Ca), 51.6 (C6) 29.1 (Cd) ppm; MS (ESI): *m/z* calcd for $C_{105}H_{133}N_7O_{28}$ $[M + H]^+$ 1940.92 found 1941.22.

***per*-(6-amino-6-deoxy)-β-CD (4)** *per*-(6-azido-6-deoxy)-β-CD (100 mg, 0.0763 mmol) was dissolved in 10 mL of MeOH and Pd/C (20 mg) suspended in H_2O (2 mL) was added. The reaction was carried out under magnetic stirring in a professional SynthWave MW (120 W) at 70 °C for 2.5 h with H_2 (10 bar). Solvents were partially evaporated and the solution was acidified with HCl to pH = 4.0. The solid was filtered, washed and the solution was lyophilized. The desired product was obtained in 91% yield (78 mg).

4 was an off-white powder; analytical data were in accordance with reported values.[Errore. Il segnalibro non è definito.]

General procedure for the synthesis of *per*-(6-ureido/thioureido-6-deoxy)-β-cyclodextrin derivatives

per-(6-amino-6-deoxy)-β-CD, as chloride salt, (0.0723 mmol) was dissolved in DMF (1 mL) and isocyanate or isothiocyanate (3.61 mmol) was added. The reaction was carried out under magnetic stirring in a MW reactor (150 W) at 85 °C for 4 h. After concentration under vacuum to half volume and the addition of acetone (10 mL), a solid product was collected by filtration on a Hirsch funnel. The precipitate was purified by reverse phase column chromatography (H_2O/CH_3OH gradient from 95:5 to methanol 100%) and the desired product was recovered. When the reaction was performed in the SynthWave MW reactor, 20 bar of nitrogen pressure were applied.

***per*-(6(3-phenylureido)-6-deoxy)-β-CD (5a)** is a white powder; yield = 38%, ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76-8.68 (s, 7H, N*H*), 7.55-6.80 (m, 35H, H-Ar), 6.70-6.32 (m, 7H, N*H*), 6.05-5.89 (m, O(2)H, O(3)H), 4.99 (br s 7H, H-1), 3.78-3.67 (m, 14H, H-3, H-5), 3.52-3.21 (m, overlapped with water, , H-2, H-4, H-6) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.6 (C=O), 140.5 (C_{ipso}), 128.9 (C_{meta}), 122.3 (C_{para}), 118.3 (C_{ortho}), 102.3 (C1), 83.5 (C4), 72.5 (C3),72.2 (C2), 70.1 (C5), 39.9 (C6), ppm; MS (ESI): *m/z* calcd for $C_{91}H_{112}N_{14}O_{35}$ $[M + H]^+$ 1961.74 found 1961.82.

***per*-(6(3-cyclohexylureido)-6-deoxy)-β-CD (5b)** is a white powder; yield = 35%, ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.97-6.64 (m, 14H, N*H*), 6.08-5.60 (m, O(2)H, O(3)H), 4.97 (br s 7H, H-1), 4.12-3.16 (m, overlapped with water, H-2, H-3, H-4, H-5, H-6, C*H*NH), 1.90-1.01 (m, 70H, C*H*$_2$ cyclohexane) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 157 (C=O), 101.7 (C1), 82.3 (C4), 71.1 (C3), 70.6 (C2), 69.6 (C5), 52.7 (*C*H cyclohexane), 42.5 (C6), 32.5 (*C*H$_2$ cyclohexane), 25.6 (*C*H$_2$ cyclohexane), 24.2 (*C*H$_2$ cyclohexane) ppm; MS (ESI): *m/z* calcd for $C_{91}H_{154}N_{14}O_{35}$ $[M + 2H]^{2+}$ 1002.54 found 1002.73.

***per*-(6(3-benzylthioureido)-6-deoxy)-β-CD (6a)** is a yellowish powder; yield = 37%, ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (s, 7H, N*H*), 7.47-7.10 (m, 42H, N*H*, H-Ar) 5.63-5.54 (m, O(2)H, O(3)H), 5.05-4.54 (m, 21H, H-1, Ph-C*H*$_2$), 4.15-3.63 (m, 21H, H-3, H-5, H-6), 3.58-3.31 (m, overlapped with water, H-2, H-4), ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.8 (C=S), 139.2 (C_{ipso}), 128.1 (C_{meta}), 127.3 (C_{ortho}), 126.9 (C_{para}), 101.7 (C1), 83.1 (C4), 72.7 (C3), 71.7 (C2), 69.5 (C5), 47.4 (Ph-*C*H$_2$), 44.3 (C6), ppm; MS (ESI): *m/z* calcd for $C_{98}H_{126}N_{14}O_{28}S_7$ $[M + 2H]^{2+}$ 1086.34 found 1086.52.

***per*-(6(3-phenylethylthioureido)-6-deoxy)-β-CD (6b)** is a white powder; yield = 36%, ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70-7.06 (m, 59H, H–Ar, N*H*), 6.20-5.73 (m, O(2)H, O(3)H), 4.88 (br s 7H, H-1), 3.86-2.62 (m, overlapped with water, H-2, H-3, H-4, H-5, H-6, C*H*$_2$NH, C*H*$_2$Ph) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.3 (C=S), 139.6 (C_{ipso}), 128.5 (C_{meta}), 102.2 (C1), 83.9 (C4), 72.9 (C3), 72.6 (C2), 47.2 (*C*H$_2$NH), 45.1 (C6), 40.48 (Ph-*C*H$_2$) ppm; MS (ESI): *m/z* calcd for $C_{105}H_{142}N_{14}O_{28}S_7$ $[M + 2H]^{2+}$ 1136.41 found.

***per*-(6(3-butylthioureido)-6-deoxy)-β-CD (6c)** is a yellowish powder; yield = 39%, ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.67-7.13 (m, 14H, N*H*), 6.18-5.48 (m, O(2)H, O(3)H), 4.89 (br s, 7H, H-1), 4.22-2.92 (m, overlapped with water, H-2, H-3, H-4, H-5, H-6, C*H*$_2$NH), 1.89-1.31 (m, 42H, NH-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_3$), 0.90 (s, 21H, C*H*$_3$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 183.4 (C=S), 102.6 (C1), 88.3 (C4), 73.3 (C3), 72.7 (C2), 70.4 (C5), 44.3 (NH-*C*H$_2$-CH$_2$-CH$_2$-CH$_3$), 43.7 (C6), 25.1 (-*C*H$_2$-CH$_2$-CH$_3$), 20.1 (-*C*H$_2$-CH$_3$), 14.4 (*C*H$_3$) ppm; MS (ESI): *m/z* calcd for $C_{77}H_{140}N_{14}O_{28}$ S_7 $[M + Na]^+$ 1955.80 found 1955.82.

Acknowledgements

This work was funded by the Compagnia di San Paolo - University of Turin (Progetti di Ricerca di Ateneo 2011, grant D15E11001710003). Authors also acknowledge ARCADE project (FP7-REGPOT-2009-1 Support action - Grant Agreement N° 245866) for the personnel exchange's financial support

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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